The nature and distribution of attraction-spheres and centrosomes in vegetable cells.[1]

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WITH PLATE XXXIII.

Introduction.

The question as to the nature of centrosomes and attraction-spheres and their importance in the cell is still in dispute. Some hold that these bodies are only temporary accumulations of the cytoplasm of the cell, while others contend that they are permanent organs, which are secondary in importance only to the nucleus itself. Moreover, the number of these bodies in each cell, their movements and manner of division, their action during impregnation of the ovum, whether they remain on the outside or inside of the resting nucleus, and even their action during the process of indirect cell division are all questions more or less in dispute. It seems, however, that from the chaos of opinions enough truth can be discovered to enable one to arrive at a safe conclusion in regard to many questions relating to them. Since the work hitherto done on plants was more especially in connection with reproductive cells, it was my purpose to work entirely with purely vegetative cells,—to study the existence of centrosomes and attraction-spheres in these cells, to find whether they remain on the outside of the resting nucleus or are included by the nuclear membrane, to determine the number of these bodies in each cell, and to trace them from the resting nucleus through the stages of karyokinesis. In my investigations, I had the assistance of Professor F. C. Newcombe, to whose suggestions are largely due whatever success I may have attained.

Historical.

Discovery.—To Professor E. van Beneden (26)[1] belongs the honor of having discovered the attraction-sphere. In the year 1887, he found in the fertilized ovum and the blastomeres of Ascaris megalocephala, at the poles of the nuclear spindle,

[1]Contribution from the Botanical Laboratory of the University of Michigan.
[1]The numbers refer to the bibliography at the close of the paper.

definite spheres each with a dense center, which he considered as permanent cell organs in connection with the nucleus. In the following year Boveri (1) observed the sphere and its center. He called the dense central body the centrosome, and regarded it as a contribution from the spermatozoon to the attraction-sphere of the ovum.

Distribution.—Investigations were made on various kinds of sexual cells until the year 1891, when Flemming (5) first found the attraction-spheres and centrosomes in the resting stages of leucocytes and in the epithelial cells of the lungs of the salamander. In the same year Guignard (10) demonstrated the existence of these bodies in reproductive vegetable cells, both in the resting stage and during karyokinesis. Since that time they have been found in the cells of many kinds of tissues, and especially in the ova of various animals.

Heidenhain (14) found them in the leucocytes of the salamander, in the medullary cells of the bones of young rabbits, and in the alveolar epithelium and leucocytes of the lung of a pneumonic patient; Bürger (3), in the proboscis-sheath of nemerteans, in resting cells; van der Stricht, (29) in the blastomeres of Triton and in the cartilaginous cells of several amphibia; E. de Wildeman (32), in Spirogyra and in the spore-mother-cells of Equisetum; Bütschli (4), in Surirella; and Schottländer (25), in the antheridia of Gymnogramme and in the spermatozoids and the ova of Chara. Heidenhain (15½) in a recent investigation has made a special study of centrosomes in the lymph cells (lymphocytes) and giant cells (megacaryocytes) from the bone marrow of the rabbit. He found them also in the spleen of the rabbit, and in the lymphatic gland and the wall of the intestine of the dog. Thus the bodies have been demonstrated in reproductive cells of both plants and animals, and also in non-reproductive animal cells.

General description.—The general appearance of a centrosome and its surrounding attraction-sphere is described by van Beneden (28) as a dense "cytocenter" around which may be distinguished a medullary and a cortical zone concentric to the central corpuscle. Heidenhain (15) also lays emphasis on the fact that the attraction-sphere is sharply limited from the surrounding protoplasm, and in many cases shows a distinct radiate structure. Guignard (10) says, in regard to the bodies seen by him in plant cells, that the attraction-spheres were composed of transparent granular areas in which the

centrosomes lay. Attraction-spheres and centrosomes vary in size in different kinds of cells. Flemming found them very small in the epithelial cells of the salamander's lungs, while in leucocytes they were much larger. Bütschli (4) says that the centrosomes, observed by him in Surirella, were so large that they were visible as a dark round granule even in the living cell.

There was a divergence of opinion, almost from the beginning, as to the number of these bodies in each cell. Many observers claimed that there was but one in the resting cell, and that this one divided before the nucleus began to divide; while others held that there were two to each resting nucleus, and that each of the two divided during nuclear division, so that each daughter nucleus was again provided with two. Flemming (5), in 1891, found the bodies double much more often than single, and he thought that where only one was seen the other might be hidden. Heidenhain (15), in 1892, stated that the number of centrosomes with each resting nucleus is always two. Guignard (10) also found them always double. Bürger, van Beneden, and van der Stricht evidently hold the opinion that there is only one. Thus there is room for doubt as to whether there may not be variation, in some tissues but one and in some two for each resting cell.

But Heidenhain (15½), in 1894, found in many cases along with the two centrosomes a third body, and sometimes a fourth, which he regards as an accessory centrosome (Nebenkörperchen). That is, he thinks that the accessory centrosome is nothing else than a centrosome of the smallest kind, which has its origin from one of the larger centrosomes. In the giant cells from the bone-marrow of the rabbit he found large numbers of centrosomes grouped together, sometimes as many as 135 in a group. There is generally one main group of these bodies in each cell, with one or more smaller accessory groups.

The bodies have been found quite universally in the same positions as regards the nucleus. In the resting cell they generally lie in a depression of the nucleus, close together, while during mitosis they are at the poles of the spindle. But Hansemann (12), while he holds that the centrosomes are permanent organs, believes with O. Hertwig (13) that they are in the nucleus during its resting stage and only come out in the first stages of division.

Activity and function.—According to Guignard (10), at the beginning of nuclear division spheres migrate to the poles of the future nuclear spindle and then each one divides during the prophase of nuclear division. But according to those who hold that there is only one attraction-sphere to the resting nucleus, the division takes place before the migration. Van der Stricht (29) finds that the division in the egg of Triton is, as a rule, effected in the quiescent stage of the nucleus, rarely during the anaphase, and exceptionally during the metaphase.

According to Heidenhain (15½) every centrosome arises from another one, not by self-division, but by budding, the largest centrosome in a group being the oldest, and the smallest the youngest.

The origin of the attraction-sphere and centrosome in the fertilized ovum does not seem as yet clearly worked out. As already stated, in the year 1888 Boveri advanced the opinion that the centrosome was brought into the attraction-sphere of the ovum along with the spermatozoon. But Guignard (10) found in the cells of the embryo-sac of Lilium Martagon that the attraction-sphere contained a centrosome before fertilization; so the hypothesis of Boveri must be given up. According to Guignard (11) there is a union of the attraction-spheres and their contained centrosomes accompanying the conjugating nucleus of the pollen-tube, with those of the nucleus of the embryo-sac during fertilization. He says that in angiosperms the two spheres brought with the male nucleus unite with the two of the female during the fusion of the two nuclei, leaving the new nucleus with two spheres, each composed of a male centrosome and its sphere united with similar bodies from the female.

Immediately, when van Beneden had made the discovery of attraction-spheres, he advanced an hypothesis as to their nature and distribution. According to van Beneden's hypothesis, there is in the cell outside of the nucleus a permanent cell organ—the attraction-sphere with its centrosome. This organ propagates itself by division when the cell does, but the division of the sphere precedes that of the cell. The rays of the spindle are attached to the sphere and are contractile fibers which attach themselves to the chromosomes and draw their halves towards the poles. The contractile rays of the spindle obtain a firm hold, for the spheres are held in place

by the cytoplasmic threads of the polar radiations. Thus an important part of the karyokinetic process would take place outside of the nucleus. Van Beneden also made the generalization that the spheres with their central bodies were of quite general distribution in both animal and vegetable cells.

Heidenhain (15½) considers that the attraction-sphere is not a constant characteristic of the cell but, as is the case in leucocytes, it is present only during the resting period of the cell, and not during the process of karyokinesis; thus the attraction-sphere is not considered to be an organ in the exact meaning of the word. He considers that the "microcentrum" (centrosome with its envelopes) of the higher organisms corresponds to the paranucleus of the protozoa while the nucleus corresponds to the macronucleus. He gives some important discussions on the physiological rôle of centrosomes and the law of their position, together with other theoretical views, but since they are beyond the scope of this paper they will not be considered here.

Bürger's (2) views are the following: He thinks that the bodies are not permanent organs, but that they are simply due to certain mechanical processes; that the central body is not the cause but the result of polar attraction. That is, he thinks the microsomes are attracted toward the center of the polar region from the periphery, and since they are solid bodies, if they are attracted equally from all sides, they form a hollow sphere which is the attraction-sphere.

Watase (31) has advanced an hypothesis somewhat similar. He thinks that the centrosome is simply a large microsome formed at the point where the greatest number of cytoplasmic filaments meet; that a barrel-shaped spindle possesses several independent microsomes at each pole instead of one centrosome. But this explanation, as well as that of Bürger, corresponds to so few of the observed facts that it seems entirely improbable. It surely could not be a reasonable explanation of the two bodies seen beside the resting nucleus by Guignard and others, nor the four spherical bodies, which can be so easily seen during metakinesis in cells of the ovary of Lilium and other plants.

For the filamentary structure of cytoplasm has not yet been demonstrated in plants; and if the centrosomes are only large microsomes, then the spindle must be divided into halves to produce the two centers at the poles, or else there must be a

crossing of filaments below the two centrosomes, neither of which has been observed.

If the views of those who hold van Beneden's hypothesis are correct, it becomes evident that every centrosome with its attraction-sphere must arise from a previous one, all the centrosomes in an organism arising from the primary one in the ovum, or according to the view of Guignard, from the two that are in the fertilized ovum, each of which represents the union of a male centrosome with one from the female. And thus they can be traced backward or forward from one generation to another the same as the nucleus.

The question naturally arises as to whether these bodies are present in cells which divide by amitosis, and if present what their action is during the process. Flemming (8) states that in leucocytes, where division is both direct and indirect, the "central bodies" are present; but they do not seem to be implicated in the fragmentation or direct division of the nucleus. He does not state what becomes of the spheres when fragmentation takes place, but concludes that only the products of karyokinetic division continue to live and multiply. Neves (19) has worked upon this subject with the spermatogonia of the salamander. He reports some discoveries, which, if they can be substantiated, truly present some very wonderful phenomena. He says that he saw the attraction-sphere become oblong, and that in various stages of the constriction of the nucleus the elongated attraction-sphere was twined in a ring about the constriction. In some cases the two ends of the elongated body appeared as though they were not yet fused together. When the division was complete, the elongated body appeared like a ring lying between but to one side of the two daughter nuclei; but there was only one of these bodies to the two nuclei. No further observations were made in regard to the subsequent action of the body and the two daughter nuclei; so the question of attraction-spheres in relation to amitotic division is yet in a very unsatisfactory state.

Plant cells especially.—The work hitherto done with plants is as follows: Guignard's investigations stand as one of the most important contributions to the subject. Guignard (10) found the attraction-spheres and centrosomes both in resting and dividing pollen-mother-cells of Lilium, Fritillaria, Listera, and Najas; in the mother-cells of the embryo-sac, with nuclei both at rest and in stages of division; in the cells of the

female apparatus derived from this nucleus; and in the endosperm. He found them in the microsporangium of Isoetes, and in the sporangium of Polypodium and of Asplenium. In his more extensive report (11) he adds many new and interesting facts, giving numerous illustrations of the appearance of these bodies in Lilium Martagon, Listera ovata, Lecojum vernum, and Galanthus nivalis. Bütschli found very large centrosomes in Surirella, a large form of diatom. E. de Wildeman (32) has found the attraction-spheres and centrosomes in Spirogyra jugalis and nitida, and in the spore-mother-cells of Equisetum, both in resting and division stages of the nucleus. Schottländer (25) claims to have found centrosomes in the male sexual cells of Marchantia polymorpha, but no attraction-spheres surrounding them. He found the attraction-spheres in the antheridia of Gymnogramme chrysophylla, and in the spermatozoids and the ova of Chara foetida. Thus in the reproductive cells of plants, and those directly concerned, the presence of attraction-spheres and centrosomes has been quite generally demonstrated, but has been reported in vegetative cells in but two cases.

Investigation.

The present work on centrosomes and attraction-spheres was begun in November, 1893. The growing tips of roots were principally used, though investigations were also made on other plant tissues. All of my material which needed sectioning was prepared according to the ordinary methods, by imbedding in paraffin and afterwards staining the sections on the slide; though I also did some staining *in toto*. After quite extensive experimenting, several methods were found of advantage in studying these bodies. Hermann's method, as given in Dr. A. Zimmermann's "Die botanische Mikrotechnik" 1892, was used very successfully on the root tips of *Allium cepa* L. The centrosomes are stained very black while the attraction-sphere is often quite clear, though sometimes somewhat stained by the safranin. The dark granular limiting layer is well defined, while the surrounding cytoplasm is red. The method is as follows: Fix the objects for one or two days in a solution of fifteen parts one per cent. platinum chloride, one part acetic acid, two to four parts two per cent. osmic acid, eighty parts water. Now wash the objects in flowing water, harden gradually in alcohol, and after that

place them from twelve to eighteen hours in pyroligneous acid. Next place the objects in a solution made of one part twenty per cent. hæmatoxylin, ninety-nine parts seventy per cent. alcohol. Keep in the dark and leave from twelve to eighteen hours, and after that in the dark for some time in seventy per cent. alcohol. Imbed and section. After the sections are fastened to the slide, cover them with a solution of potassium permanganate, which has so much water that it possesses a light rose color, and leave until they have an ocher color. Then wash the sections with a solution of one part hydric oxalate, one part potassic sulphate, 1,000–2,000 parts water. After this, stain the sections for three to five minutes in a saturated alcoholic (100%) solution of safranin; clear and mount in Canada balsam. I also prepared root tips in Flemming's fixing fluid, and after imbedding and sectioning, stained first with Kleinenberg's hæmatoxylin and then with a two per cent. aqueous solution of acid fuchsin. The ovaries and anthers of *Lilium longiflorum* Thunb., I stained in several ways. The centrosomes and attraction-sphere will be stained quite well, however, by simply leaving them for a considerable length of time in anilin-safranin, and then taking out the excess of color with alcohol. By another method I took equal parts of an aqueous (two per cent.) solution of acid fuchsin and acetic methyl-green, which in some cases made a very favorable stain.

The last method tried was one suggested to me by Professor Newcombe: (1) a one per cent. aqueous solution of ferrous sulphate, (2) a five per cent. aqueous solution of tannic acid, (3) anilin-safranin (one part of one per cent. alcoholic solution of safranin with two parts water), (4) an aqueous solution of picro-nigrosin, strong enough to have a dark bluish-green color. The slides holding the sections were placed thirty to forty-five minutes in the iron solution, then washed in water; next, the same length of time in the tannin, and washed again. Now the sections were covered again with the iron solution and left for a minute or two or until they changed to a rather dark color. After washing off the iron in a stream of water they were stained in the anilin-safranin from thirty minutes to one hour, and afterwards fifteen minutes or more in the picro-nigrosin. After raising them through the grades of alcohol and being careful so as not to take out too much of the safranin stain, they were mounted in balsam. The centrosomes were stained very dark and the attraction-spheres well

defined, often showing the radiate structure. Any of the above methods will give fairly good results with plant cells, if proper care is taken; but I prefer Hermann's method or the iron-tannin-safranin stain as giving the best results.

I found the bodies as a rule very small and used continually a Zeiss $\frac{1}{12}$ immersion lens, and generally a no. 8 compensating ocular. As a general rule, I think more difficulties are encountered in studying these bodies in plants than in animals. The killing fluids do not penetrate so readily, which causes more displacement and distortion of the elements of the cell. In the vegetable cell, moreover, are generally present large numbers of chromatophores, starch grains, crystalloids, and other such bodies, which may greatly interfere with the identification of such small bodies as centrosomes. In the root tips which I studied, I found the leucoplasts a constant source of trouble; and many stains which might otherwise be very useful become worthless because of the readiness with which they color these bodies. The radiate structure of the cytoplasm is also much less marked than in animal cells.

I found centrosomes and attraction-spheres in the following named material: in the young root tips of *Allium cepa* L. (figs. 1–11), in resting cells and the various stages of karyokinesis; in the root tips of *Vicia faba* L. (fig. 15); in the root tips of *Tradescantia rosea* L. (fig. 16); in the resting cells of the epidermis of the old bulb scales of *Allium cepa* L. (figs. 17 and 18); and in the epidermis of the anther (fig. 14) and the walls of the ovary of *Lilium longiflorum* Thunb. (figs. 12 and 13).

In the onion root tips, I was able to trace them through nearly all the stages of nuclear division (figs. 1–10) as well as in the young ovaries of Lilium. In the other material I did not trace them through the whole series, but in the Vicia and Tradescantia root tips I was able to see them in several of the stages of karyokinesis. In the epidermal cells of the lily anther, I found them quite common in cells that were completely in the resting stage (fig. 14); while in the epidermis of the onion scales, where all the nuclei were in the resting stage and where there is no subsequent cell-division, I also succeeded in demonstrating the existence of these bodies in many cases (figs. 17 and 18).

Whenever I observed the bodies in resting cells, there were always two centrosomes, each with an attraction-sphere, which was in most cases marked off from the surrounding cyto-

plasm by a well defined granular layer; and in cases where karyokinesis had advanced to any considerable extent, two centrosomes could be distinguished at each pole of the spindle. Sometimes there appeared to be but one at each pole, but careful focusing generally demonstrated the fact that one was lying below the other. In the root tips of Allium, where the division is tranverse to the axis of the root when one goes a little distance from the apex, the attraction-spheres always appear at the upper or lower end of the nucleus as seen in longitudinal section. In the resting cells, they generally lie quite close to the nucleus in a little indentation.

In the epidermis of the onion scales I observed these bodies in a sufficient number of cases to convince me that they were true attraction-spheres, since they had the same appearance and took the same stain as those which I saw by the side of the close skein of the daughter nucleus. Now in these epidermal cells of the onion scales the nuclei are all resting, and therefore the objection that the centrosomes may have just come out of the nucleus in the beginning of division cannot be made; and so I hold that the attraction-spheres with their centrosomes do not enter the nucleus during its resting stage but remain permanently outside of the nuclear membrane. Moreover, these cells of the epidermis of the bulb scales of Allium were all definitive resting cells; yet with the iron-tannin-safranin stain it was demonstrated that the centrosomes and attraction-spheres were still present beside the nucleus, and that they retained their usual structure.

When division of the nucleus takes place, I found the attraction-spheres in the onion roots at the very beginning of the close mother skein stage, one at the upper and one at the lower pole of the future spindle, still close to or in contact with the nucleus (fig. 2); and though I did not find any stage where one of the bodies had gone only part of the way around, yet there can be no doubt that one or both had traveled around from their original position to the poles. In the following stages the spheres elongate, and generally by the time when the nucleus has reached the loose mother skein (fig. 3) the centrosomes and their spheres have divided, though they still lie closer together than in the later stages. During metakinesis and the daughter star stage (figs. 5–7), they can be seen very distinctly at each pole; and they keep this position in relation with the nucleus through all the suc-

ceeding stages of the division (figs. 8–10), and through the resting stage of the nucleus until a new division of the nucleus takes place.

It will be seen from the fact that the centrosomes remain at the position of the pole of the daughter nucleus until the division following, that in the case where the next spindle is in the same direction as the preceding one, one of the centrosomes must travel through 180° to come to the opposite pole of the nucleus. But in the case where the division is at right angles to the preceding one, each centrosome must travel through 45° in order to reach the poles of the future spindle. Now in the Allium root tips, in many cases, in a given chain of cells, division will take place logitudinally at a certain distance from the apex; and from that point onward there will be two chains of cells instead of one, and farther on the division of the nucleus will again be in a plane transverse to the axis of the root. Thus taking such an example where the cell has divided transversely, if the next division is longitudinal each centrosome must pass through 45°; the next division being transverse again both bodies must again travel as before; but in the third division one of the bodies will be stationary while the other passes through 180°. In a strand of cells coming from the apex of the root, the cells as they continue to divide always maintain a curve, and the attraction-spheres will not be quite 180° apart as they lie at the two poles of a dividing nucleus. I have observed in cells at these points, that the spheres lay inclined with the daughter nucleus toward the concave side of the strand of cells. The bodies do not always travel in the same direction, as will readily be seen when we take into consideration a strand of cells from an onion root whose elements are dividing transversely. If the spheres are at the proximal end of the nucleus when division occurs, the migrating one will travel in a direction toward the apex of the root; but if they lie at the distal side of the nucleus it must travel in the opposite direction. The whole subject shows that the centrosomes with their spheres travel in a very complicated manner during the formation of any given vegetable tissue.

A very peculiar phenomenon was noticed in the root tips of Allium. In many cases the spindle was formed obliquely in the cell, the attraction spheres lying near opposite corners of the cell as it appeared in longitudinal section (fig. 11).

The actual length of the spindle from pole to pole was greater than the length of the cell. It appeared as though there was not enough room for the division of the nucleus, and the bodies had wandered to the corners in order to gain more space for the formation of the spindle. It seems to me that this phenomenon explains itself if we admit that the attraction-spheres are directive in their function, and control nuclear division; but the appearance might be just as well accounted for by supposing that the controlling power rested in the nucleus or the cytoplasm of the cell in general.

Results.

The special results of the investigation are as follows:

1. Centrosomes aud attraction-spheres are present in non-reproductive as well as reproductive vegetable cells.

2. They remain on the outside of the nucleus during its resting stage.

3. They persist in cells which have ended their growth and division.

Besides the foregoing results, the present investigation furnishes confirmation to the following propositions:

1. In phanerogams there are two of these bodies for each resting nucleus.

2. When the nucleus begins to divide, one or both of the bodies migrate so as to take their positions at the poles of the future spindle.

3. Subsequently they immediately begin to divide, the division being completed in the prophase of the mother nucleus.

4. After their migration, the attraction-spheres remain at the poles of the nuclear spindle, and do not change their position until the beginning of the following division.

5. They seem to be organs which institute and direct nuclear division.

Summary.

The theory advanced by Van Beneden has received the support of many of the leading biologists, and has with some additions been quite generally substantiated by investigations. Taking the facts and opinions of those who have studied these bodies, into general consideration, the subject seems to be in the following condition: There is a permanent body in the

cell—the attraction-sphere with a centrosome—which is of universal distribution in both plants and animals—at least in all cells which divide by karyokinesis. This body propagates itself by division. As a rule, there seem to be two of these bodies for each resting nucleus, but in some cases only one. They remain constantly ouside of the nucleus. They appear to be the organs which direct nuclear division. It seems that there is a union of the attraction-spheres and centrosomes accompanying the male nucleus with those of the female nucleus during impregnation of the ovum. The bodies migrate and divide, and are thus carried from one cell to the other throughout the entire organism, whether plant or animal.

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Explanation of Plate XXXIII.

Figs. 1-11, *Allium cepa*—root tips.

Fig. 1. Resting nucleus, showing two attraction-spheres with controsomes.—Fig. 2. Beginning stage of division, one attraction-sphere being at e ch pole. The centrosomes are slightly elongated.—Fig. 3. Loose mother skein; the attraction-spheres have completely divided —Fig. 4. Formation of the nuclear spindle; the attraction-spheres and centrosomes lie at the poles.—Fig. 5. Metakinesis; the bodies were seen only at one end of the spindle.—Fig. 6. Metakinesis farther advanced; the attraction-spheres with their centrosomes appear at each pole of the spindle, and are surrounded by the cytoplasmic radiations. —Fig. 7. Daughter star; at the upper end of the spindle one of the centrosomes is displaced.—Fig. 8. Loose daughter skein, showing the four attraction-spheres.—Fig. 9. Close daughter skein, only the upper nucleus showing the bodies.—Fig. 10. Daughter nucleus nearly complete. The nuclear membrane

has appeared. The two centrosomes with their spheres appear at the upper side.—Fig. 11. A cell in which the spindle lies obliquely.

Figs. 12–14, *Lilium longiflorum.*

Fig. 12. Last stage of metakinesis, showing the attraction-spheres and centrosomes, with cytoplasmic radiations around the poles. (From wall of young ovary.)—Fig. 13. Last stage of close daughter skein; the upper daughter nucleus shows attraction-spheres and centrosomes. (From wall of ovary.)—Fig. 14. Resting nucleus from the epidermis of the anther, with two attraction-spheres.

Fig. 15. *Vicia faba*—root tips. Daughter star; showing two centrosomes with attraction-spheres at the upper end of the spindle.

Fig. 16. *Tradescantia rosea*—root tips. Close daughter skein; the centrosomes with spheres appear at the lower side.

Fig. 17. *Allium cepa*—epidermis of bulb scales. Resting nucleus with two attraction-spheres and centrosomes.—Fig. 18, similar to fig. 17.